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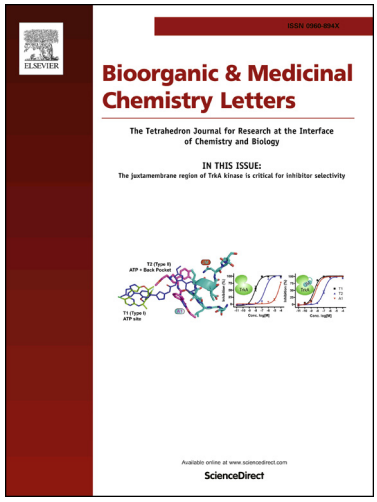

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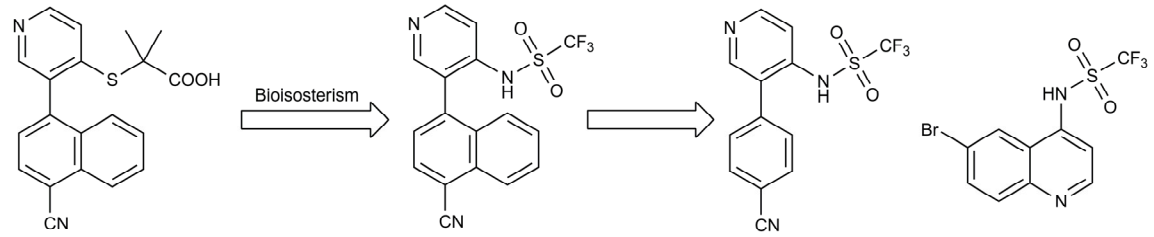

4
Verinurad

9b
IC_{50} = 10 nM

16i
IC_{50} = 2 nM

19b
IC_{50} = 83 nM

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Synthesis and evaluation of sulfonamide derivatives as potent Human Uric Acid Transporter 1 (hURAT1) inhibitors

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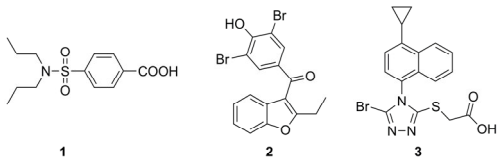
ABSTRACT

This letter presents synthesis and structure-activity relationship study of sulfonamide derivatives as inhibitors of Human Uric Acid Transporter 1 (hURAT1). Among all tested sulfonamide derivatives, compounds **9b**, **16i** and **19b** exhibited excellent inhibition activity with IC_{50} value of 10, 2, and 83 nM, respectively. In addition, compounds **9b** and **19b** demonstrated moderate PK profile in rats.

In humans, the final purine metabolism product is urate (uric acid) and predominately excreted via the kidney; as humans are uricase deficient, they cannot metabolize urate to the allantoin.[1-2] Urate serves as a biologically radical scavenger and antioxidant in humans, whereas the high levels of urate leads to hyperuricemia which causes many diseases such as gout, hypertension, chronic kidney diseases, cardiovascular diseases, and metabolic syndrome.[3-4] In humans, the elevated levels of serum urate results from the absence of uricase and the presence of effective renal urate reabsorption.[5-6] Therefore, the renal reabsorption process plays a pivotal role in the elevated levels of plasma urate. In the process of reabsorption, urate is reabsorbed from the tubular lumen across the apical membrane into the proximal tubular cells and then extruded from the cells across the basolateral membrane into the blood. In the kidneys (i.e. proximal tubular cells), the Human Uric Acid Transporter 1 (hURAT1, encoded by SLC22A12) is localized at the apical membrane and recognized as a major transporter protein for urate reabsorption.[7-8] Therefore, inhibition of hURAT1 would be a very effective way to treat hyperuricemia.

To date, several commercially available drugs inhibiting urate uptake mediated by hURAT1, including probenecid **1**, benzbromarone **2** and lesinurad **3** have been marketed (**Fig. 1**). Benzbromarone **2**, a potent hURAT1 inhibitor, was withdrawn from the market of many countries in 2003 because of acute liver failure, but it is still available in some European countries, as well as in several Asian countries and South American countries.[9] Lesinurad **3** is an oral hURAT1 inhibitor developed by AstraZeneca and approved as combination therapy with a xanthine oxidase inhibitor, Allopurinol or Febuxostat, for the treatment of hyperuricemia by the Food and Drug Administration (FDA) in 2015.[10-11]



1 Probenecid **2** Benzbromarone **3** Lesinurad

Figure 1. hURAT1 inhibitor drugs

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In recent years, intense efforts to develop hURAT1 inhibitors are ongoing in pharmaceutical industry and academia, and different kinds of compounds are emerging. Most of these compounds have a carboxylic acid group moiety. For example, carboxylic acid compounds **4** and **5** developed by AstraZeneca could lower serum urate levels through inhibition of hURAT1 with nanomolar potency (**Fig. 2**).[12-14] In addition, diverse hURAT1 inhibitors (**6**-**8**) from patents and literatures also contain carboxylic acid moiety (**Fig. 2**).[15-17]

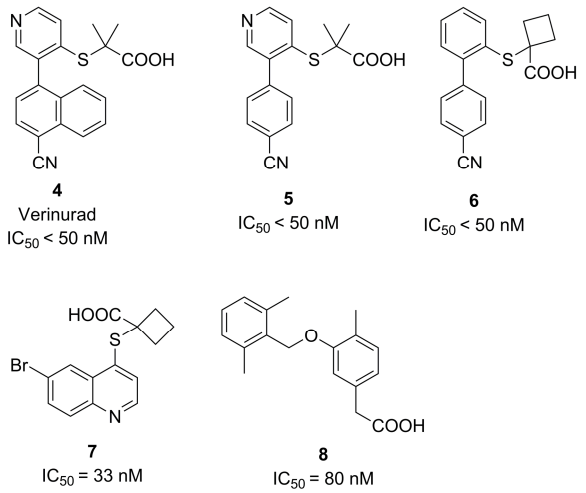
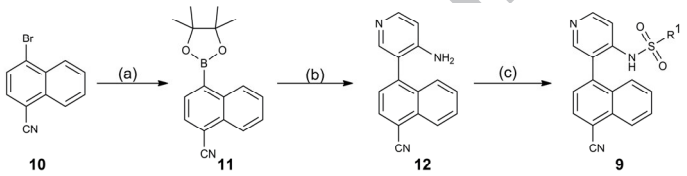


Figure 2. hURAT1 inhibitors with carboxylic acid moiety.

Due to the lack of structural information of hURAT1, it is difficult for the structure-based drug design approach to guide the lead identification effort. It is found that uric acid is a substrate of organic anion transporters such as hOAT1 and hURAT1, and predominates in the anionic form in blood and urine.[18] Therefore, it is assumed that a potent hURAT1 inhibitor may need a negative charge to interact with the positively charged binging pocket.[19-20] The carboxylic acid group in many hURAT1 inhibitors may serve as a negative charge to achieve the inhibitory activity. It is well known that bioisosterism could be used as a tool to modify the key compounds into more potent agents. Replacement of carboxylic acid group with proper bioisosteres that replace the only hydroxyl portion or the entire carboxylic group has been utilized widely.[21] Hence, we decided to replace the carboxylic acid of hURAT1 inhibitor **4** with nonclassical bioisostere sulfonamide to probe its influence on the inhibition activity (**Fig. 3**).

Figure 3. Sulfonamide replaced the carboxylic acid portion of hURAT1 inhibitor **4**.

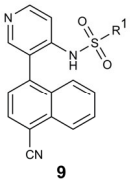
Initially, we synthesized the methanesulfonamide compound **9a** by following **Scheme 1**. Bis(pinacolato)diboron reacted with **10** under the catalyst of $Pd(dppf)Cl_2$, which gave compound **11**. Further suzuki coupling of **11** with 4-amino-3-bromopyridine provided **12**, which subsequently reacted with methanesulfonyl chlorides under the conditions of Et_3N in DCM to afford compound **9a**. It was found that methanesulfonamide compound **9a** showed the moderate activity with an IC_{50} value of 261 nM, which suggested that sulfonamide moiety of compound **9a** had the similar biological activity with the carboxylic portion of Verinurad **4**. Encouraged by the result, further optimization of the sulfonamide moiety could be performed. Hence, a series of sulfonamide compounds were synthesized by following **Scheme 1** and the IC_{50} values of them were listed in **Table 1**.



Scheme 1. Reagent and conditions: (a) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, H_2O, 100□; (b) 4-amino-3-bromopyridine, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, H_2O, 90□; (c) R^1SO_2Cl or Tf_2O, DCM, Et_3N.

Table 1

Activity of compounds with different sulfonamide moieties.



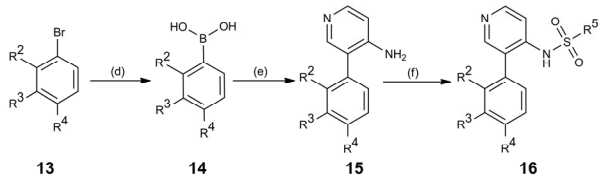
compounds	R^1	hURAT1 IC_{50} [a] (nM)
9a	Me	261
9b	CF_3	10
9c	Et	713
9d	n-Pr	1100
9e	i-Bu	597
9f	Ph	127
9g	p-fluorophenyl	2000
9h	p-cyanophenyl	1400

[a] IC_{50} values are the means of two determinations.

As shown in **Table 1**, compound **9b** showed excellent inhibition activity with IC_{50} value of 10 nM, which was 26-fold improvement in potency in comparision with the **9a**. The phenyl analogue **9f** displayed slightly better activity than **9a**. Whereas other aliphatic alkylsulfonamide compounds **9c**, **9d** and **9e** were much less effective. It could be explained that the trifluoromethyl, a strong electron-withdrawing group, made the sulfonamide more acidic, which helped IC_{50} value boost from 261 nM of **9a** to 10

nM of **9b**. Surprisingly, compounds **9g** and **9h** exhibited lower inhibition activity than **9f**.

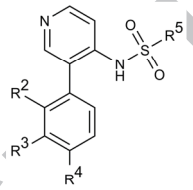
Next, we investigated the influence of different aromatic rings on the inhibition activity. The compounds containing different aromatic rings were synthesized by following **Scheme 2** and the IC_{50} values were listed in **Table 2**. As shown in **Table 2**, when R^5 was kept as methyl group, it was found that the compounds showed low inhibition activity when there were substituents on *ortho* position of benzene ring such as compounds **16a** and **16b**. When the substituents were on the *meta* position of benzene ring such as compounds **16c** and **16d**, it was found that these compounds exhibited slightly better inhibiton activity. However, when the substituents were attached to *para* position of benzene ring such as **16e** and **16f**, we found that compound **16f** with nitrile group on *para* position displayed slightly higher activity than naphthalene analogue **9a**. Surprisingly, when R^5 was changed from the methyl to trifluoromethyl, it was found that compounds **16g**, **16h** and **16i**, showed much better activity regardless of aromatic rings. It was noted that compound **16i** exhibited very strong inhibition activity with an IC_{50} value of 2 nM.



Scheme 2. Reagent and conditions: (d) n-BuLi, THF, trimethyl borate, -78□, 2N HCl; (e) 4-amino-3-bromopyridine, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, H_2O, 90□; (f) R^5SO_2Cl or Tf_2O, DCM, Et_3N.

Table 2

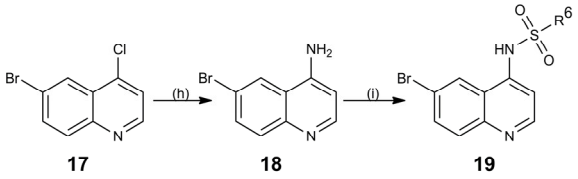
Activity of sulfonamide compounds with different aromatic rings.



compounds	R^2	R^3	R^4	R^5	hURAT1 IC_{50} [a] (nM)
16a	OMe	H	H	Me	20900
16b	CF_3	H	H	Me	11999
16c	H	OMe	H	Me	9836
16d	H	F	H	Me	2827
16e	H	H	CF_3	Me	4059
16f	H	H	CN	Me	203
16g	OMe	H	H	CF_3	241
16h	H	H	OMe	CF_3	215
16i	H	H	CN	CF_3	2
16j	H	H	CN	Ph	390
16k	H	H	CN	p-fluorophenyl	4000

[a] IC_{50} values are the means of two determinations.

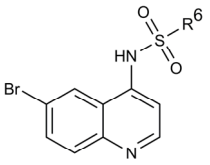
Driven by a desire to further demonstrate sulfonamide could replace the carboxylic acid group and maintain or improve the inhibition activity of hURAT1. Besides the aryl substituted pyridine scaffold described above, we also applied the sulfonamide approach to quinoline, a 2,3-benzopyridine scaffold. Methanesulfonamide **19a** and trifluoromethanesulfonamide **19b** were synthesized as shown in the **Scheme 3** and their IC_{50} values were listed in **Table 3**. The IC_{50} value suggested the compound **19b** with an IC_{50} value of 83 nM was compared favorably with the reported carboxylic acid compound **7** with an IC_{50} value of 33 nM.



Scheme 3. Reagent and conditions: (h) acetamide, K_2CO_3, 180□; (i) R^6SO_2Cl or Tf_2O, DCM, Et_3N.

Table 3

Activity of sulfonamide compounds containing quinoline scaffold



compounds	R^6	hURAT1 IC_{50} [a] (nM)
19a	Me	3600
19b	CF_3	83

[a] IC_{50} values are the means of two determination

Compounds **9a**, **9b**, **16i** and **19b** were tested in male Sprague-Dawley rats to evaluate their pharmacokinetics and the results were shown in **Table 4**. The pharmacokinetic parameters, such as $T_{1/2}$ and AUC_{0-t}, were calculated via noncompartmental model using WinNonlin6.2.1 software program. By oral dose of 10 mg/kg, we found that half-life was improved from 3.2 h (**9a**) to 19.6 h (**9b**), and body exposure (AUC) of trifluoromethanesulfonamide **9b** was 2-fold improvement in comparison with methanesulfonamide **9a**. However, for the benzene analogue **16i**, half–life was reduced to 4.1 h and body exposure (AUC) was only 6.4μg·h/ml in comparison with **9b**. The **19b**, quinoline as scaffold, not only gave the highest exposure (AUC, 181μg·h/ml) but also exhibited lowest plasma

clearance ($T_{1/2}$, 19.8 h) among **9a**, **9b**, **16i** and **19b** by oral dose of 10 mg/kg.

Table 4

Pharmacokinetic properties of compounds **9a**, **9b**, **16i**, and **19b**

Parameters	Rat(PO)				Rat(IV)			
	9a	**9b**	**16i**	**19b**	**9a**	**9b**	**16i**	**19b**
Dose (mg/kg)	10	10	10	10	1	1	1	1
T_{max} (h)	1	1	2	5.3	0.9	0.9	0.9	0.9
$T_{1/2}$ (h)	3.2	19.6	4.1	19.8	3.9	10.8	4.4	14
C_{max} (μg/ml)	17.4	9.0	0.3	10.5	5.7	8.1	2.6	7.7
AUC_{0-t} (μg·h/ml)	38.6	72.1	6.4	181	6.5	33.1	2.4	64
MRT (h)	3.3	27.2	5.2	28.7	2.7	13.8	1.9	19
Cl (ml/min/kg)	ND[a]	ND	ND	ND	2.5	0.3	6.8	0.2
Vss (L/kg)	ND	ND	ND	ND	0.4	0.3	0.8	0.2
F (%)	59	21.8	27	28	ND	ND	ND	ND

[a] ND: No Data

In summary, we found that the sulfonamide could be utilized to improve or maintain the hURAT1 inhibition activity by the bioisosteric replacement of carboxylic group. Especially, triflourosulfonamide **9b**, **16i**, and **19b** exhibited excellent in vitro inhibition activity. Through pharmacokinetic studies of compounds **9a**, **9b**, **16i** and **19b**, we found that **9b** and **19b** showed moderate pharmacokinetic profile.

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Supplementary data

Supplementary data (experimental procedures and biological measuring methods) associated with this article can be found.

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Highlights

- Sulfonamide showed the hURAT1 inhibition activity.
- Structure-activity relationship of sulfonamide as inhibitors of hURAT1.
- Compounds **9b** and **19b** demonstrated moderate PK profile in rats.